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EFFECTS OF COTTON PLANT ALLELOCHEMICALS AND NUTRIENTS ON BEHAVIOR AND DEVELOPMENT OF TOBACCO BUDWORM

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Abstract—Female moths of the tobacco budworm, *Heliothis virescens* (F.), oviposit in the terminals of the cotton plant, *Gossypium hirsutum* (L.). The hatched larvae migrate to the terminal area and then to small squares (buds), on which they feed, finally burrowing into the anthers where they grow and develop. They attempt to avoid gossypol glands as they feed. Chemically related evidence explains, in part, these observations. The calyx crown of resistant lines (which is avoided) is high in the terpenoid aldehydes (TAs) including gossypol. HPLC data showed that the gossypol content of both susceptible and resistant glanded lines is equal, while the hemigossypolone and heliocides H_1 and H_2 are greatly increased in resistant lines and presumably are more closely associated with resistance. Analysis for total amino acids in cotton square tissues showed that there was a gradation from the calyx and calyx crown, which were lowest, to the anthers, the site of final insect development, which were highest. Synthetic diets mimicking amino acid distribution in anthers were found to be successful for larval growth and development.

Key Words—Tobacco budworm, *Heliothis virescens*, Lepidoptera, Noctuidae, cotton, *Gossypium hirsutum*, plant resistance, plant-insect interaction, terpenoids, gossypol, allelochemicals.

INTRODUCTION

The female moths of the tobacco budworm, *Heliothis virescens* (F.), oviposit in the terminals of the cotton plant, *Gossypium hirsutum* L. There is no evi-

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dence that they are specifically attracted to the terminals by chemical cues, but rather that they are most proximate to the terminals during an overflight. Most of the eggs are placed singly on the small terminal leaves approximately 12 cm^2 in size (Ramalho, 1983). Three days after oviposition, the eggs hatch. Immediately after hatch, the young larvae will feed on the leaf tissue for a brief period before they migrate into the terminal area, which is comprised of meristematic tissue (immature squares and leaves). The young larvae spend about three days feeding on small squares with the potential of destroying a maximum of four squares each during this feeding period (Parrott, unpublished data).

During this period, gossypol is toxic to the larvae (Hedin et al., 1988), which also are observed to avoid consuming glands that contain gossypol. However, when the larvae molt into the second instar at about 72 hr, they can nonselectively consume the glands (Parrott et al., 1983). Earlier, Shaver and Parrott (1970) reported young larvae to be more sensitive to gossypol than older larvae. This finding was later supported by laboratory studies in which gossypol and two other allelochemicals were fed in diets to 1-, 3-, and 5-day-old tobacco budworm (TBW) larvae. All three allelochemicals were toxic to 1- and 3-day-old larvae, but they were not toxic to 5-day-old insects. Evidence was obtained that the insects biosynthesized detoxifying enzymes, mixed function oxidases (MFOs), because piperonyl butoxide, a known inhibitor of MFOs, inhibited growth when added to the diets (Hedin et al., 1988).

When the larvae leave the terminal, they move onto small squares that arise at position one of the fruiting branch (Ramalho et al., 1984). They then prefer to feed along the margin area of the calyx crown. Using laboratory feeding tests in which larvae fed on squares from several experimental lines, Parrott et al. (1989) found that they fed less on squares from plants that have high gland density in the calyx crown, a normally preferred feeding site of young larvae.

During the later second and third instars, the larvae burrow through the calyx or petals of the cotton squares into the anthers. Once they reach the anthers, their growth rate immediately accelerates. Work by Shaver et al. (1977) showed that the major source of nutrients for tobacco budworm larvae in detached cotton buds is in the anthers. The nutritional requirements, however, have never been defined chemically, in part because the tobacco budworm has never been reared on synthetic amino acid diets. However, the expectation is that if this could be achieved, it would provide the basis for more reliable evaluation of the effects of natural and synthetic constituents.

The objectives of this study were to determine whether the migration of the tobacco budworm from the terminal leaf, terminal area (meristematic tissue), calyx crown, and finally to the anthers can be at least partly explained by, or associated with, the gradient concentrations of allelochemicals and nutrients.

METHODS AND MATERIALS

Cultivars and Agronomic Practices. In 1988, three lines with demonstrated resistance to the TBW—DH-118, DH-126, and DH-118 × ST 213 H—were planted on the Plant Science Farm, Mississippi State, Mississippi. For comparison, four susceptible lines, DH-118 × ST-213 L, ST-213, ST-825, and ST-213 gl, were also planted. Not all lines were used in every test. Standard cultural practices were followed during the growing season, except that some of the replicated plots were not protected from insects. All plant material was collected from cotton plants of similar maturity.

Gathering and Processing of Plant Tissue. Terminal leaves, squares, and square parts were harvested, frozen, freeze-dried, and then ground through a 40-mesh screen. The powders were stored in vials at −20°C until they were evaluated. A portion of the freshly gathered squares was dissected into the following parts: anthers, petals, bracts, calyx, and calyx crown. These tissues were also freeze-dried, ground to a powder, and stored at −20°C to await subsequent evaluations.

Insects and Diets. Neonate tobacco budworm larvae used in the various tests were obtained from a colony at the Crop Science Research Laboratory, Mississippi State, Mississippi, maintained as described by Jenkins et al. (1982). Larvae (20 per test) in some tests were placed in 30-ml clear plastic cups containing 10 g/cup of a Nutrisoy–wheat germ diet (Bioserv, Frenchtown, New Jersey).

In tests where the synthetic amino acids replaced the Nutrisoy–wheat germ protein as a source of nitrogen, the amino acids were procured either from Bioserv, or from Sigma Chemical Co. (St. Louis, Missouri). The amino acid distribution and levels in these diets were based on protein and amino acid analyses of the soy flour, wheat germ, and cotton anthers (methods and results described later in the text). The nonprotein dietary ingredients [and a separate vitamin mixture (Pack C)] were also provided as dry mixes by Bioserv. The dietary ingredients are listed in Table 1.

Analytical Procedures. The Association of Official Analytical Chemists (AOAC) method (Horwitz, 1975) for total protein, 2.049 (percent nitrogen × 6.25) was used to determine that the crude protein content of the Bioserv Pack B containing soy flour and wheat germ as protein sources was 22.7%; i.e., the quantity of amino acids that was required for addition to the Pack B less protein to provide an equivalent amount to that supplied by the soy flour and wheat germ.

Analysis of Allelochemicals. Analyses for gossypol and related terpenoid aldehydes were performed on cyclohexane–ethyl acetate–acetic acid (500:500:1; CHEA) extracts of plant (whole square and anther) tissue by the

TABLE 1. INGREDIENTS IN STANDARD LABORATORY AND SYNTHETIC AMINO ACID DIETS FOR TOBACCO BUDWORM

Ingredient	Percent of total content	
	Soy–wheat germ	Amino acid
Amino acids	0	18.9
Soy flour	24.8	0
Wheat germ	21.0	0
Wesson salts	0.6	0.6
Sucrose	24.8	22.3
Methyl paraben	0.8	1.3
Sorbic acid	0.8	0.4
Aureomycin, 14%	0.8	0.1
Cholesterol	0	0.1
Linseed oil	0	1.1
Corn cob grits	0	32.8
Agar	13.6	14.3
Vitamins[a]	5.8	6.1
Acids[b]	1.7	1.8

[a] Pack C from Bioserv, Inc., Frenchtown, NJ. Ascorbic acid, vitamin E acetate, biotin, calcium pantothenate, choline citrate, folic acid, inositol, niacin, pyridoxine HCl, riboflavin, thiamine HCl, vitamin B_{12} in sucrose; 1:1.
[b] Propionic acid–Phosphoric Acid: 9:1.

phloroglucinol reaction [2% in absolute EtOH-concentrated HCl (1:1)]; let stand 1 hr, with subsequent spectrometric analysis at 550 nm. The concentration was determined by comparison with data obtained from authentic gossypol and is expressed as gossypol equivalents. Condensed tannin analyses were performed on 70% aqueous methanol extracts of tissue. The anthocyanidin chromophore was developed from the tannin by boiling 1 hr with 1-butanol–HCl (95:5) (Hedin et al., 1983b). The concentration was determined by comparison with the color obtained at 550 nm from a purified cotton condensed tannin sample, the structure of which was elucidated by Collum et al. (1981). The anthocyanin content was determined by measuring the absorbancy at 540 nm of freeze-dried tissue extracted with methanol–water–HCl (79:19:3), using the molar extinction coefficient (E) of cyanidin 3-β-glucoside (Hedin et al., 1967). Flavonoids were determined after extraction of freeze-dehydrated tissue with 70% aqueous acetone. Diphenylboric acid–ethanolamine complex (Natural Product Reagent A, Aldrich Chemical Co., 1%) in methanol was added, and the chromophore absorptivity at 440 nm was determined and compared to that obtained from a purified sample of isoquercitrin, the most prevalent flavonoid in cotton.

HPLC Analyses of Terpenoid Aldehydes and Amino Acids. HPLC analysis

of the gossypol-type terpenoid aldehydes was performed on a reverse-phase C_{18} column according to the procedure of Stipanovic et al. (1988), who also kindly provided samples of hemigossypolone, and heliocides H_1 and H_2 for column calibration. Amino acids were determined by HPLC analyses after acid hydrolysis, employing their phenylthiocarbamyl derivatives (Cohen et al., 1986).

Procurement of Chemicals. All commercially available chemicals (other than the amino acids) were obtained from Sigma Chemical Co. Gossypol was a gift from the Southern Regional Research Center, USDA, New Orleans, Louisiana.

Statistical Procedures. Data obtained from the various analyses and measurements were subjected to the analysis of variance, and LSD values were calculated using SAS (1985) except for the HPLC analyses on the terpenoid aldehydes where standard errors of the mean are provided. No statistical analyses were obtained on the custom-procured amino acid data.

RESULTS AND DISCUSSION

Allelochemicals in Cotton Leaves, Squares, and Their Parts. The whole squares and square parts, anthers and stigma, bracts, calyx, calyx crown, and petals were analyzed for the major cotton allelochemicals (Table 2). The strains most resistant to TBW, DH 118 and DH 118 × ST 213 H, were highest in gossypol in the whole square and in the square parts. The DH 118 × ST 213 L strain was very similar to ST 213 in the level of gossypol and level of resistance to TBW, whereas the DH 118 × ST 213 H strain was very similar to DH 118 in the level of gossypol and the level of resistance to TBW in all square parts.

The tannins were highest in ST 213 glandless and in ST 213, the two strains most susceptible to TBW. This agrees with previous data of Hedin et al. (1983a,b) where tannin of glandless (susceptible) lines was about 20% higher than that of glanded resistant lines. The anthocyanin content should be somewhat correlated with gossypol because it often occurs as a halo around gossypol glands (Hedin et al., 1983a,b), but this was the case only in the anthers and bracts. The flavonoids were higher in petals and bracts of the resistant DH 118 and the DH × ST 213 H lines than in the DH 118 × ST 213 L and ST 213 lines. The same was true for flavonoids in whole squares.

Terpenoid Aldehydes (TAs) in Cotton Squares. Because gossypol is known to coexist in the plant with a number of related TAs, the gossypol fractions of three susceptible and two resistant double haploid lines were investigated by HPLC. Of the six major TA components listed in Table 3, the identities of four had previously been established, and their HPLC elution times were identical

TABLE 2. ALLELOCHEMICALS IN COTTON LEAVES, SQUARES, AND SQUARE PARTS OF TBW RESISTANT AND SUSCEPTIBLE LINES

Line	Percent content			
	Gossypol[a]	Tannins	Anthocyanin	Flavonoids
Terminal leaves				
ST 213 gl	0.13	8.90	0.13	4.58
ST 213	0.35	11.60	0.17	4.38
DH 126	0.39	11.67	0.19	4.42
LSD 0.05	0.02	0.65	0.02	0.62
Whole square				
ST 213 gl	0.12	10.10	0.16	2.14
ST 213	0.43	10.76	0.14	2.18
DH 118 × ST 213 L	0.48	7.51	0.11	2.04
DH 118 × ST 213 H	0.60	7.37	0.11	3.14
DH 118	0.63	6.86	0.11	2.66
LSD 0.05	0.18	0.74	0.02	0.33
Petals				
ST 213 gl	0.14	6.35	0.05	3.46
ST 213	0.69	4.55	0.05	2.09
DH 118 × ST 213 L	0.95	4.30	0.04	2.02
DH 118 × ST 213 H	1.25	4.40	0.05	2.16
DH 118	1.49	4.73	0.05	2.26
LSD 0.05	0.15	0.44	0.01	0.50
Anthers and stigma				
ST 213 gl	0.20	6.14	0.07	0.90
ST 213	1.14	5.12	0.08	0.30
DH 118 × ST 213 L	1.11	5.26	0.07	0.34
DH 118 × ST 213 H	1.37	4.56	0.09	0.30
DH 118	1.29	5.69	0.09	0.29
LSD 0.05	0.10	0.58	0.02	0.06
Bracts				
ST 213 gl	0.13	8.07	0.11	1.57
ST 213	0.13	8.37	0.17	1.33
DH 118 × ST 213 L	0.13	5.38	0.12	1.30
DH 118 × ST 213 H	0.22	6.56	0.17	1.42
DH 118	0.22	6.49	0.15	1.38
LSD 0.05	0.02	0.37	0.02	0.08
Calyx crown[b]				
ST 213 gl	0.09			
ST 213	0.12			
DH 118 × ST 213 L	0.11			
DH 118 × ST 213 H	0.12			
DH 118	0.15			
LSD 0.05	0.02			
Calyx[b]				
ST 213 gl	0.09			
ST 213	0.11			
DH 118 × ST 213 L	0.11			
DH 118 × ST 213 H	0.14			
DH 118	0.31			
LSD 0.05	0.02			

[a] Gossypol analyzed by the phloroglucinol procedure.
[b] Insufficient calyx tissue precluded analyses for tannins, anthocyanin, and flavonoids.

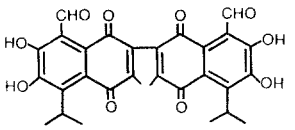
with those of available authentic samples (gossypol, hemigossypolone, and heliocides H_1 and H_2). Furthermore, the identity of the eluates was confirmed by EI-MS via solid probe analyses.

Tentative identifications of the other two maxima were obtained via EI-MS analysis. Comparison of the eluate at 8.2 min with that of an authentic

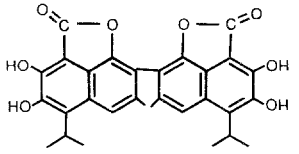
TABLE 3. TERPENOID ALDEHYDE CONTENT (%) IN SELECTED LINES OF COTTON SQUARES AND LEAVES

Line	Percent content						
	HGQ	GQ	GL	G	H_1	H_2	Total[a]
Buds							
ST-213 gl	0.01	0.02	0.01	0.02	0.01	0.01	0.08 ± 0.02
ST-213	0.04	0.09	0.03	0.14	0.06	0.07	0.43 ± 0.04
ST-825	0.02	0.08	0.03	0.15	0.04	0.05	0.37 ± 0.03
DH-118	0.14	0.06	0.03	0.12	0.17	0.11	0.63 ± 0.07
DH-126	0.16	0.07	0.03	0.14	0.20	0.13	0.73 ± 0.06
Leaves							
ST-213	0.10	0.02	0.01	0.03	0.12	0.12	0.40 ± 0.04
DH-118	0.17	0.03	0.02	0.05	0.16	0.18	0.61 ± 0.05

[a] Mean ± standard error of the mean.



a. Gossypolone



b. Gossypol Lactone

FIG. 1. Structures of gossypolone and gossypol lactone.

sample of gossypolone (Figure 1a) prepared by us (Phillips and Hedin, 1990) showed that the fragmentation patterns were nearly identical.

EI-MS analysis of the maximum at 10.7 min gave an apparent $[M]^+$ of 514. The most prominent ion fragments were as follows: *m/z* 514(100), 499(75), 482(65), 465(35), and 439(15). Repeated attempts to isolate sufficient quantities of this component for NMR and IR by liquid chromatography using silica gel and Sephadex LH-20 were unsuccessful because of degradation.

It was proposed that the apparent molecular ion could be accounted for if the gossypol aldehydic functions were oxidized to the dicarboxylic acid and subsequently dehydrated to form the dilactone. Therefore, gossypol was oxidized by stirring in air with MnO_2 in acetone or benzene. Both preparations dehydrated rapidly to the presumed dilactones, without the use of a dehydrating agent, and gave fragmentation patterns essentially identical to that of the isolate. Again, efforts to prepare sufficient samples in pure form for spectral work were unsuccessful. The presumed structure (Figure 1b) is 6,6′,7,7′-tetrahydroxy-3,3′-dimethyl, 5,5′-bis(methylethyl)-2,2′-binaphthalene-8,8′-γ-dibutyrolactone. The trivial name "gossypol lactone" is suggested.

The total TA content was lower for each of the three susceptible lines than in the two resistant lines (Table 3). It is noteworthy that the gossypol content was essentially the same in squares of all of the glanded lines (0.12–0.15%). This suggests that resistant and susceptible lines both biosynthesize equal (basal) amounts of gossypol, and thus resistance is determined by other factors, perhaps other terpenoid aldehydes.

Hemigossypolone (Table 3) accounts for less than 10% of the TAs in squares of susceptible lines but over 20% of the TAs in resistant lines. The heliocides H_1 and H_2 are also increased sharply in resistant lines (two to fourfold). On the other hand, neither square gossypolone or gossypol lactone (M^+514) is increased above the "basal" levels in resistant lines. While they, along with gossypol, can be presumed to contribute some resistance, they do not appear to account for the increased resistance of the DH lines.

Gossypol lactone is probably an artifact arising from the oxidation and subsequent lactonization of gossypol during processing and/or storage. The distribution of TAs in leaves (Table 3) is somewhat different. The gossypol content is low compared to hemigossypolone and heliocides H_1 and H_2 in both a susceptible and a resistant line. The latter three are again higher in the resistant line than the susceptible line as compared with gossypol.

Chan et al. (1978), Stipanovic et al. (1977, 1978), and Hedin et al. (1983a,b) determined the inhibition of TBW larval growth expressed as ED_{50}, percent of diet, for several TAs including gossypol, hemigossypolone, and heliocide H_1. They averaged about 0.10%. Therefore, at least when reconstituted in diets, they have about the same level of toxicity. If this relationship

also exists in the plant sites that are encountered by the insect, resistance as mediated by TAs is related to the concentration in the tissue of importance.

Relationships of Cotton Plant Amino Acids to TBW Growth and Development. For background information, the total amino acids, free and bound, were determined by HPLC analysis (Cohen et al., 1986). Amino acid analysis data for ST 213 leaves, squares, and their parts; ST 213 gl anthers; DH 118 calyx crown; and the TBW Pack B (soy flour plus wheat germ) are summarized in Table 4. The total amino acid contents of calyx and calyx crown are the lowest (13.1% and 14.8%). Bracts and petals are intermediate (17.0% and 17.3%), and anthers are highest (22.5% and 23.7%). It is of apparent significance that the larvae migrate to, and complete their growth and development in, the tissue of highest protein content.

The total sugar content of anthers is known to be high relative to other square tissues and also is higher in lines susceptible to boll weevil oviposition (Hedin and McCarty, 1990). Nutritionally, therefore, anthers appear to be the most favorable cotton tissue development site for insects.

The distribution of the amino acids in the various tissues is similar, and the ratio of essential to nonessential amino acids (45–47%) has been found adequate for larval growth of the fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] (Hedin et al., 1990). The only likely growth constraints may be that methionine (6.8%) in DH 118 calyx crown and arginine (9.9%) in Pack B are high relative to the other essential amino acids for optimum growth. The high arginine of Pack B is contributed mostly by the wheat germ. Wheat germ amino acids, as a synthetic amino acid diet, failed to support fall armyworm larval growth (Hedin et al., 1990). Analysis of cotton plant amino acids has been reported on numerous occasions in the literature, including Parrott et al. (1969) and Lindig et al. (1980). The results are similar.

Tables 5 and 6 provide information about the synthetic amino acid contents of diets that were formulated and used to investigate whether they could successfully support growth and development of TBW larvae. Using synthetic amino acids as the sole source of nitrogen is not novel. Evidently the first report of the use of synthetic amino acids for the growth of a cotton insect was that of Vanderzant (1965) for the boll weevil *Anthonomus grandis* Boh. Dadd (1985) reported in a review article on the rearing of several other insects on amino acid diets. We recently reported on the rearing of the fall armyworm with synthetic amino acid diets (Hedin et al., 1990). The replacement of plant protein with amino acids in diets being tested for effects of allelochemicals removes from conjecture the possibility that some unknown factor, perhaps bound to the protein, also may be affecting growth and development.

In these tests (Table 5) amino acid diets were formulated at the same nitrogen level supplied by the protein (diets 1 and 4), at a level increased 50% to

TABLE 4. AMINO ACIDS IN SELECTED COTTON PLANT TISSUES AND TBW LABORATORY DIET

Amino acid	Amino Acids (% of total)									
	Terminal leaves, ST 213	Squares, ST 213	Calyx crown, ST 213	Calyx, ST 213	Bracts, ST 213	Petals, ST 213	Anthers, ST 213	Anthers, ST 213 gl	Calyx crown, DH 118	TBW Pack B
Arginine	7.3	6.0	7.9	6.6	7.4	7.2	6.9	6.6	7.0	9.9
Histidine	1.9	3.5	1.8	2.0	2.0	2.1	2.0	2.0	1.8	2.1
Isoleucine	5.5	5.5	5.3	5.0	5.4	4.6	5.5	5.5	4.8	4.5
Leucine	8.8	9.1	7.2	7.4	9.0	7.2	7.8	7.7	7.9	7.3
Lysine	7.1	8.6	8.5	6.9	7.1	6.5	6.8	6.9	7.2	6.4
Methionine	2.9	3.0	2.8	2.8	2.8	2.4	2.8	2.8	6.8	2.8
Phenylalanine	5.6	4.7	5.0	4.5	5.8	4.2	4.4	4.4	4.7	4.9
Threonine	4.0	4.2	4.2	3.2	3.9	3.3	4.7	4.7	4.4	3.5
Tryptophan[a]	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Valine	6.4	6.8	6.2	5.7	6.3	5.4	5.6	5.7	5.5	5.2
Alanine	7.0	6.1	6.4	6.4	6.9	6.0	5.6	5.7	5.7	5.4
Aspartic acid	8.3	10.3	11.2	15.5	8.2	17.7	11.1	11.3	11.5	11.0
Cysteic acid	1.8		0.3	1.9	1.8	1.9	0.2	0.2	0.2	0.3
Cystine	0.2	0.8	0.1	0.2	0.2	0.4	0.3	0.3	0.2	0.3
Glutamic acid	12.4	12.9	12.4	12.9	12.5	12.3	12.3	11.2	11.9	17.4
Glycine	5.9	6.0	5.6	5.1	5.8	4.9	5.1	5.1	5.2	4.8
Proline	6.3	5.7	5.8	6.3	6.3	5.8	8.1	7.5	5.6	5.6
Serine	3.9	3.5	4.8	3.9	3.9	3.9	5.5	5.5	5.0	4.5
Tyrosine	3.3	1.9	3.1	2.3	3.3	2.8	3.9	3.5	3.2	2.7
Amino acids in sample (%)	17.4	18.7	14.8	13.1	17.3	17.0	23.7	22.5	17.4	26.8

[a] Analysis for tryptophan was obtained for ST 213 squares only and included as an estimate of its content in the other tissues.

TABLE 5. FORMULATIONS OF SYNTHETIC AMINO ACID DIETS FOR TBW BASED ON ANALYSIS OF CONTENTS IN COTTON ANTHERS AND LABORATORY SOY–WHEAT GERM DIET[a]

	Cotton anthers			Soy–wheat germ		
Amino acid	g/14.01 g	g/21.01 g	g/14.16 g	g/14.01 g	g/21.01 g	g/14.35 g
Diet	1	2	3	4	5	6
Arginine	0.92	1.38	0.92	1.43	2.15	1.43
Histidine	0.28	0.42	0.28	0.31	0.47	0.31
Isoleucine	0.78	1.17	0.78	0.66	0.99	0.66
Leucine	1.12	1.68	1.12	1.06	1.59	1.06
Lysine	0.95	1.43	0.95	0.92	1.38	0.92
Methionine	0.14	0.21	0.28	0.06	0.09	0.31
Phenylalanine	0.63	0.95	0.63	0.70	1.05	0.70
Threonine	0.66	0.99	0.66	0.50	0.75	0.50
Tryptophan	0.20	0.30	0.28	0.19	0.29	0.31
Valine	0.81	1.22	0.81	0.76	1.14	0.76
Alanine	0.81	1.22	0.81	0.78	1.17	0.78
Aspartic acid	1.79	2.69	1.79	1.60	2.40	1.60
Cysteic acid	0.03	0.05	0.03	0.04	0.06	0.04
Cystine	0.04	0.06	0.06	0.06	0.09	0.06
Glutamic acid	1.88	2.82	1.88	2.55	3.83	2.55
Glycine	0.71	1.07	0.71	0.70	1.05	0.77
Proline	1.16	1.74	1.16	0.81	1.22	0.81
Serine	0.77	1.16	0.77	0.66	0.99	0.66
Tyrosine	0.52	0.78	0.52	0.41	0.62	0.41

[a] Diets 2 and 5 were increased in the amino acid content by 50%; diets 3 and 6 were increased only with methionine and tryptophan.

determine whether the amino acid concentration was limiting (diets 2 and 5), and at the base level of diets 1 and 4 but with increased amounts of methionine and tryptophan whose analysis was thought to be low. Diets 1–3 were based on the distribution of amino acids in cotton anthers, and diets 4–6 were based on the distribution in the soy–wheat germ diet (Table 4).

Tables 1 and 6 give the ingredients of the formulated diets. Table 6 also lists larval weights at nine days, pupal weights, and days to pupation, along with the statistical information. As expected, insects fed the standard laboratory diet grew more rapidly and pupated sooner. However, insects on the three cotton anther amino acid diets (diets 1–3) achieved pupation having pupal weights only somewhat lower than those on the standard diet. The days to pupation were longer. Increasing the level of amino acids by 50% (diet 2) had an adverse effect compared to diet 1. Increasing the methionine and tryptophan (diet 3) improved the growth rate through nine days, but the improvement was not sus-

TABLE 6. TBW GROWTH AND DEVELOPMENT ON SYNTHETIC AMINO ACID DIETS BASED ON CONTENT IN COTTON ANTHERS AND LABORATORY SOY-WHEAT GERM DIET

Ingredient	Cotton anthers			Soy-wheat germ			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7[a]
Pack B (g)	0	0	0	0	0	0	61.8
Amino acids (g), Pack B	14.01	21.02	14.35	14.01	21.02	14.16	0
Less protein (g)	48.0	48.0	48.0	48.0	48.0	48.0	0
Agar (g)	10.6	10.6	10.6	10.6	10.6	10.6	10.6
Vitamins (g)	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Acids (ml)	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Hot water (ml)	265	265	265	265	265	265	265
Cold water (ml)	177	177	177	177	177	177	177
Larval weight, 9 days, mg; LSD = 13.4	5.8 C[b]	8.9 B	33.9 B	0.6 C	0.7 C	34.9 B	317.7 A
Pupal weight, mg: LSD = 9.3	261.5 B	235.1 D	230.6 D	[c]	[c]	247.3 C	310.0 A
Days to pupation, LSD = 0.4	21.0 D	23.4 A	22.7 B	[c]	[c]	21.9 C	14.5 E

[a] Diet 7 is the standard laboratory diet.
[b] Means followed by the same letter are not significantly different.
[c] No survival.

tained through pupation. Larval growth on the soy-wheat germ-based amino acid diets 4 and 5 did not occur. With added methionine and tryptophan (diet 6), however, acceptable growth and development took place.

The growth lag can perhaps be explained by the need for insects to develop mechanisms to transport and metabolize the free amino acids. The somewhat poorer growth observed on the cotton anther-based amino acid diet 2 can probably be explained by the high amino acid-carbohydrate ratio. Phytophageous insects have been reported to grow best on diets in which the protein and carbohydrates each contribute about one third of the nutrients (Dadd, 1985). The poorer performance of the soy-wheat germ diets relative to the cotton anther diets may be in part explained by the high arginine content of diets 4–6.

The overall performance of these amino acid diets is similar to that observed with the fall armyworm (Hedin et al., 1990). In these present tests, all 10 amino acids reported as essential for rats were included in each of the tests. Dadd (1985), in reviewing the literature, reported that for specific insects additional

amino acids were found to be essential for insect growth. As previously stated, the growth and development observed in these tests was not as rapid as with the standard diet, but presumably could be optimized if desired. However, the present results established that the TBW can be reared successfully on synthetic amino acid diets, evidently for the first time. Moreover, the establishment of effective amino acid diets means that the effects of allelochemicals can be evaluated free from the effects of undefined growth factors.

SUMMARY

Chemical evidence has been obtained that explains, in part, observations about the migration of tobacco budworm larvae from terminal leaves to the square, their avoidance of calyx crowns that are high in gossypol glands, and their burrowing into the anthers where they develop.

The calyx crown of resistant lines was found to be higher in terpenoid aldehydes than in susceptible lines. HPLC data showed that the gossypol content of both susceptible and resistant lines is equivalent (basal), while the hemigossypolone and heliocides H_1 and H_2 are greatly increased in resistant lines and presumably are more closely associated with resistance.

Analysis for total amino acids in cotton square tissues showed that there was a gradation of levels from the leaves, calyx, and calyx crown, which were low; to the petals and bracts, which were intermediate; and to the anthers, the site of final development, which was highest. Amino acid diets based on their distribution in anthers were found to be successful for larval growth and development.

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